

REMARKS

Claims 81, 83, and 87-92 are allowed. Claim 93 is cancelled without prejudice or disclaimer. Claims 94-99 are added.

New claim 94 recites that the parent alpha amylase has an A domain, a B domain and a C domain, in which the A domain has an amino acid sequence corresponding to residues 1-103 and 206-395 of SEQ ID NO:2 and has the three-dimensional structure of a beta/alpha 8 barrel with 8 central beta strands and 8 flanking alpha-helices; wherein said B domain has an amino acid sequence corresponding to residues 104-205 of SEQ ID NO:2 and has a three-dimensional structure of a 5-stranded anti-parallel beta-sheet structure containing at least one long loop structure and having connectivity -1, +3, -1X, and +2; and wherein said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID NO:2 and has a three-dimensional structure of beta-strands which forms a single 8-stranded sheet structure. Support for claim 94 is found, inter alia, in the specification at page 7, line 31 to page 9, line 28.

New claims 94 also recites that the three-dimensional structure has at least three calcium ion binding sites and one sodium ion binding site, wherein two bound calcium ions form part of a linear cluster of three bound ions, wherein the central bound ion of the linear cluster is a sodium, and wherein the linear cluster is located between the A domain and the B domain, and wherein a third calcium binding site is located between the A domain and the C domain. Support for claim 94 is found at page 9, line 30 to page 10, line 4, and at page 10, lines 15-16.

New claims 95-99 recite that the parent alpha amylase has an amino acid sequence having at least 75%, 80%, 85%, 90% and 95% homology to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4 or SEQ ID NO:6. Support for claims 94-99 is found in the specification as originally filed at page 5, lines 4-15.

Accordingly, claims 81, 83 and 87-99 are pending.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the above amendments and the following remarks is requested.

I. The Rejection of Claims 93 under 35 U.S.C. 112

Claim 93 is rejected under 35 U.S.C. 112, first paragraph, specifically it is stated:

“...because the specification, while being enabling for a method for producing a variant of a parent α -amylase having an amino acid sequence that is at least 70% homologous to the sequences of SEQ

ID NO's: 2, 4 or 6 comprising the the step of generating a model of said parent α -amylase using a three-dimensional structure of α -amylase having the amino acid sequence of SEQ ID NO:13 and having the coordinates shown in Appendix 1, does not reasonably provide enablement for a method for producing a variant of a parent α -amylase comprising the step of generating a model of said parent α -amylase using a three-dimensional structure for SEQ ID NO:13 shown in Appendix 1 adapted to said parent α -amylase. "

According to the office action, the specification does not enable any skilled person in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention in scope with these claims. The office action specifies:

"...in order to practice methods of claim 93 one skilled in the art would need an x-ray crystallographic three-dimensional structure of an α -amylase having amino acid sequence of SEQ ID NO:13 having coordinates shown in Appendix 1 and the means and ways to adapt said coordinates."

Further, the office action states:

"...it is not apparent whether said programs [*i.e. protein model-building computer software*] are readily available to the public and what parameters and settings have been used for modeling TERM and BAN. Moreover, there is no guidance as to how to carry out such modeling with any other parent α -amylase having less homology to SEQ ID NO: 2, 4 or 13 than TERM and BAN."

Claim 93 is also rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject-matter which applicant regards as his invention, because

"...the specification does not teach the steps and parameters involved in the step of adaption rendering the metes and bounds of the claim unascertainable".

The cancellation of claim 93 renders these rejections moot. However, if applicable to the new claims, Applicants submit the following remarks with respect to the enablement of new claims 94-98.

Prior to the present invention, the three-dimensional structures of only very few alpha-amylases had been solved. A three-dimensional structure of a Termamyl-like bacterial alpha-amylases did not exist at the time. In fact, the structure of Termamyl-like amylases had mistakenly been assumed to be similar to those of other known non-Termamyl-like amylases. Attempts to build structure models of Termamyl-like amylases based on the known non-Termamyl-like structures were in retrospect doomed to failure due to this erroneous assumption.

The present inventors determined the first actual three-dimensional structure of a Termamyl-like bacterial alpha-amylase as shown in the specification. Surprisingly, this structure was found to have, among other unique features, a special domain structure in loop 3 of the A domain. This special domain structure denoted the B domain was previously unknown and not suggested in the art.

The characteristics for the three-dimensional structure of SEQ ID NO. 13 are proposed by Applicants to be representative of all Termamyl-like alpha-amylases (see the specification at page 11, Ins. 20-22). These characteristics include the atomic coordinates of SEQ ID NO:13 as depicted in Appendix 1, as well as the other structural properties listed in the new claims submitted herewith.

Therefore, in addition to the actual three-dimensional structure for SEQ ID. NO. 13 depicted in Appendix 1, Applicants submit that they have also described (and had possession of), as discussed below, the three-dimensional structures of other homologous Termamyl-like alpha-amylases, such as, the three-dimensional structures of the amylases shown in SEQ ID. No's: 2, 4 and 6 (see the specification at page 11, Ins. 9-30; see *also* Example 1). Indeed, the atomic coordinates for the vast majority of the amino acids comprised in the amino acid sequences of the enzyme species falling within the claimed genus are virtually identical to the amino acid coordinates for SEQ ID. NO. 13 as depicted in Appendix 1.

Moreover, due to the high homology required by the claims, the remaining amino acid coordinates that are not virtually identical, are readily predicted by an artisan using the methods described in the specification and/or methods well-known in the art, as discussed below. Such prediction for amino acids sequences having high degrees of homology is routine practice in the art, and is done, for example, by aligning homologous amino acid sequences and using homology

based modeling computer programs as known in the art. An example of such homology building for *B. licheniformis* alpha-amylase is described in Example 1 of the specification.

To show the commercial availability of homology-based modeling computer programs at the time of filing, we submit herewith a copy of the first four pages and that last three pages (with references) of a User Guide (dated October 1995) to a protein model-building computer program called "Homology, release 95.0" available from Biosym/MCI, San Diego, USA. The program is still available from Accelrys Inc., San Diego, USA (formerly Biosys) as part of the InsightII software package.

The Examiner alleges, that in order to practice the claimed invention, one skilled in the art would need an X-ray crystallographic three-dimensional structure of an unspecified alpha-amylase. Applicants respectfully disagree. An X-ray crystallographic three-dimensional structure for each alpha-amylase species falling within the claimed invention (*i.e.*, as determined by crystallizing each alpha-amylase) is plainly not required to practice the claimed invention.

The crux of the present invention is, that an artisan can readily provide a suitably accurate three-dimensional model structure of an enzyme, just as soon as the first actual detailed information about the three-dimensional structure of a sufficiently homologous enzyme becomes available. For example, when the unique three-dimensional structure of the Termamyl-like alpha-amylase of SEQ ID NO:13 was identified, it immediately became possible for the artisan to build reliable three-dimensional models of homologous Termamyl-like alpha-amylases, e.g. based on the structure shown in Appendix 1, or on the other structural properties listed in the new claims submitted herewith, for instance by using modeling computer programs. The skilled artisan could then in turn use those models to modify the properties of said homologous alpha-amylases e.g. by standard methods of single-site amino acid substitution/deletion/addition.

Once the three-dimensional structure of the first Termamyl-like alpha-amylase was solved by the instant inventors, solving subsequent homologous structures became a routine straightforward task for the skilled artisan.

The skilled artisan would even be able to model sufficiently homologous enzymes by building them, in turn, upon an enzyme-model which itself was originally based on the first actual three-dimensional structure, thus completely obviating the need for the actual atomic coordinates of that first structure altogether.

In this regard, Applicants have provided that first key three-dimensional structure for a Termamyl-like alpha-amylase (Appendix 1), and have provided the scope for which this three-dimensional structure is applicable, namely, enzymes with sufficiently high homology ("at least

70%") to SEQ ID NO. 13, such as SEQ ID NO's: 2, 4 and 6. Applicants have also provided a specific example in which a three-dimensional model of an alpha-amylase which was not SEQ ID NO:13 was provided. See, e.g., Example 1 for B. licheniformis-alpha-amylase.

The level of skill in this particular art is extremely high, and therefore Applicants submit, that the skilled artisan at the time would have been readily able to build a three-dimensional model-structure of Termamyl-like alpha-amylases sufficiently homologous with SEQ ID NO: 13, based on the information in Appendix 1, or based on the other structural characteristics identified in the application, for instance by using appropriate modeling computer programs.

In addition, Applicants submit, that the skilled artisan at the time would have been able to in turn build further three-dimensional model-structures of Termamyl-like alpha-amylases sufficiently homologous with SEQ ID NO: 13, either based on a first model built on the basis of the information in Appendix 1, or based on a subsequently solved actual structure of a related Termamyl-like alpha-amylase which was made possible through the disclosure of the present invention, thereby obviating any future need for the actual structure shown in Appendix 1.

The level of skill in the art of protein modeling is exemplified by the following references (copies are submitted herewith for the Examiners convenience):

- ✓ 1) Greer J. 1990. Comparative Modeling Methods: Application to the Family of the mammalian Serine Proteases. Publishers: Wiley-Liss, Inc. Proteins: Structure, Function, and Genetics 7:317-334.
- ✓ 2) Greer J. 1985. Protein Structure and Function by Comparative Model Building. Annals of The New York Academy of Sciences, vol. 439: 4463.
- ✓ 3) Greer J. 1981. Comparative Model-building of the Mammalian Serine Proteases. J Mol Biol 153:1027-1042.
- ✓ 4) Thirup and Jones. 1986. Using known substructures in protein model building and crystallography. J EMBO 5(4):819-822.
- ✓ 5) Blundell T.L. *et al.* 1987. Knowledge-based prediction of protein structures and the design of novel molecules. Nature 326(26):347-352.

Therefore, since the present invention provided the first key or cornerstone in building three-dimensional model-structures of Termamyl-like alpha-amylases, the scope of the present claims should be commensurate with that highly significant contribution to the art, and not be limited to models derived directly from the data in Appendix 1.

For the foregoing reasons, Applicants submit that the new claims 94-98 are also in

condition for allowance, and early notice to that effect is respectfully requested.

The Examiner is hereby invited to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

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